

WHAT IS CLAIMED IS:

1. A method for detecting the presence or absence of herpes simplex virus (HSV) in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if a nucleic acid molecule encoding HSV DNA polymerase is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize within no more than five nucleotides of each other, wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of fluorescence resonance energy transfer (FRET) between said donor fluorescent moiety of said first HSV DNA polymerase probe and said corresponding acceptor fluorescent moiety of said second HSV DNA polymerase probe,

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

2. The method of claim 1, wherein said pair of HSV DNA polymerase primers comprises a first HSV DNA polymerase primer and a second HSV DNA polymerase primer, wherein said first HSV DNA polymerase primer comprises the sequence

5'-GCT CGA GTG CGA AAA AAC GTT C-3' (SEQ ID NO:1), and wherein said second HSV DNA polymerase primer comprises the sequence

5'-CGG GGC GCT CGG CTA AC-3' (SEQ ID NO:2).

3. The method of claim 1, wherein said first HSV DNA polymerase probe comprises the sequence

5'-GCG CAC CAG ATC CAC GCC CTT GAT GAG C- 3' (SEQ ID NO:3), and wherein said second HSV DNA polymerase probe comprises the sequence

5'-CTT GCC CCC GCA GAT GAC GCC- 3' (SEQ ID NO:4).

5 4. The method of claim 1, wherein said first HSV DNA polymerase probe comprises the sequence

5'-GTA CAT CGG CGT CAT CTG CGG GGG CAA G- 3' (SEQ ID NO:5), and

wherein said second HSV DNA polymerase probe comprises the sequence

5'- T GCT CAT CAA GGG CGT GGA TCT GGT GC- 3' (SEQ ID NO:6).

10 5. The method of claim 1, wherein the members of said pair of HSV DNA polymerase probes hybridize within no more than two nucleotides of each other.

6. The method of claim 1, wherein the members of said pair of HSV DNA polymerase probes hybridize within no more than one nucleotide of each other.

7. The method of claim 1, wherein said donor fluorescent moiety is fluorescein.

8. The method of claim 1, wherein said acceptor fluorescent moiety is selected from the group consisting of LC-Red 640, LC-Red 705, Cy5, and Cy5.5.

9. The method of claim 1, wherein said detecting step comprises exciting said biological sample at a wavelength absorbed by said donor fluorescent moiety and visualizing and/or measuring the wavelength emitted by said acceptor fluorescent moiety.

25 10. The method of claim 1, wherein said detecting comprises quantitating said FRET.

11. The method of claim 1, wherein said detecting step is performed after each cycling step.

30 12. The method of claim 1, wherein said detecting step is performed in real-time.

13. The method of claim 1, wherein the HSV DNA polymerase amplification product differs in sequence between HSV-1 and HSV-2 by at least one nucleotide.

5 14. The method of claim 1, further comprising determining the melting temperature between one or both of said HSV DNA polymerase probe(s) and said HSV DNA polymerase amplification product, wherein said melting temperature confirms said presence or said absence of said HSV.

10 15. The method of claim 14, wherein said melting temperature distinguishes between HSV-1 and HSV-2.

16. The method of claim 1, wherein the presence of said FRET within 10 cycles is indicative of the presence of an HSV infection in said individual.

17. The method of claim 1, wherein the presence of said FRET within 20 cycles is indicative of the presence of an HSV infection in said individual.

18. The method of claim 1, wherein the presence of said FRET within 30 cycles is indicative of the presence of an HSV infection in said individual.

19. The method of claim 1, wherein the presence of said FRET within 37 cycles is indicative of the presence of an HSV infection in said individual.

20. The method of claim 1, wherein the absence of said FRET within 37 cycles is indicative of the absence of an HSV infection in said individual.

21. The method of claim 1, wherein the presence of said FRET within 40 cycles is indicative of the presence of an HSV infection in said individual.

22. The method of claim 1, wherein the presence of said FRET within 50 cycles is indicative of the presence of an HSV infection in said individual.

23. The method of claim 1, further comprising: preventing amplification of a
5 contaminant nucleic acid.

24. The method of claim 23, wherein said preventing comprises performing said amplifying step in the presence of uracil.

10 25. The method of claim 24, wherein said preventing further comprises treating said biological sample with uracil-DNA glycosylase prior to a first amplifying step.

26. The method of claim 1, wherein said biological sample is selected from the group consisting of an ocular swab, a genital specimen, a dermal specimen, a pap smear, amniotic fluid and cerebrospinal fluid.

27. The method of claim 1, further comprising:
performing at least one cycling step, wherein said cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV TK primers to produce an HSV TK amplification product if a nucleic acid molecule encoding HSV TK is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of HSV TK probes, wherein the members of said pair of HSV TK probes hybridize within no more than five nucleotides of each other, wherein a first HSV TK probe of said pair of HSV TK probes is labeled with a donor fluorescent moiety
25 and wherein a second HSV TK probe of said pair of HSV TK probes is labeled with a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first HSV TK probe and said corresponding acceptor fluorescent moiety of said second HSV TK probe.

28. The method of claim 27, wherein said pair of HSV TK primers comprises a first HSV TK primer and a second HSV TK primer, wherein said first HSV TK primer comprises the sequence

5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G,

and wherein said second HSV TK primer comprises the sequence

5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C.

29. The method of claim 27, wherein said first HSV TK probe comprises the sequence

5'-CGC GCG ACG ATA TCG TCT ACG TAC- 3' (SEQ ID NO:9), and wherein said second HSV TK probe comprises the sequence

5'-CGA GCC GAT GAC TTA CTG GCA GGT G- 3' (SEQ ID NO:10).

30. The method of claim 1, wherein said cycling step is performed on a control sample.

31. The method of claim 30, wherein said control sample comprises said portion of said nucleic acid molecule encoding said HSV DNA polymerase.

32. The method of claim 1, wherein said cycling step uses a pair of control primers and a pair of control probes, wherein said control primers and said control probes are other than said HSV DNA polymerase primers and said HSV DNA polymerase probes, respectively, wherein a control amplification product is produced if control template is present in said sample, wherein said control probes hybridize to said control amplification product.

33. A method of distinguishing between HSV-1 and HSV-2 in a biological sample from an individual, the method comprising:

performing at least one cycling step, wherein said cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV-1 DNA polymerase amplification product if a nucleic acid molecule encoding HSV-1 DNA polymerase is present in

said sample and/or an HSV-2 DNA polymerase amplification product if a nucleic acid molecule encoding HSV-2 DNA polymerase is present in said sample, wherein said HSV-1 and said HSV-2 amplification products differ in sequence by at least one nucleotide, wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize within no more than five nucleotides of each other, wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a corresponding acceptor fluorescent moiety;

detecting the presence or absence of FRET between said donor fluorescent moiety of said first HSV DNA polymerase probe and said corresponding acceptor fluorescent moiety of said second HSV DNA polymerase probe, wherein the presence of FRET is indicative of the presence of HSV in said biological sample, wherein the absence of FRET is indicative of the absence of HSV in said biological sample; and

determining the melting temperature between one or both of said HSV DNA polymerase probes and said HSV DNA polymerase amplification products, wherein said melting temperature distinguishes between HSV-1 and HSV-2.

34. An article of manufacture, comprising:
a pair of HSV DNA polymerase primers,
a pair of HSV DNA polymerase probes, and
a donor fluorescent moiety and a corresponding acceptor moiety.

35. The article of manufacture of claim 34, wherein said pair of HSV DNA polymerase primers comprises a first HSV DNA polymerase primer and a second HSV DNA polymerase primer, wherein said first HSV DNA polymerase primer comprises the sequence 5'-GCT CGA GTG CGA AAA AAC GTT C-3' (SEQ ID NO:1), and wherein said second HSV DNA polymerase primer comprises the sequence 5'-CGG GGC GCT CGG CTA AC-3' (SEQ ID NO:2).

36. The article of manufacture of claim 34, wherein said first HSV DNA polymerase probe comprises the sequence

5'-GCG CAC CAG ATC CAC GCC CTT GAT GAG C-3' (SEQ ID NO:3), and wherein said second HSV DNA polymerase probe comprises the sequence

5'-CTT GCC CCC GCA GAT GAC GCC-3' (SEQ ID NO:4).

37. The article of manufacture of claim 34, wherein said first HSV DNA polymerase probe comprises the sequence

5'-GTA CAT CGG CGT CAT CTG CGG GGG CAA G- 3' (SEQ ID NO:5), and

wherein said second HSV DNA polymerase probe comprises the sequence

5'- T GCT CAT CAA GGG CGT GGA TCT GGT GC- 3' (SEQ ID NO:6).

38. The article of manufacture of claim 34, wherein said pair of HSV DNA polymerase probes are labeled with said donor fluorescent moiety and said corresponding acceptor fluorescent moiety.

39. An article of manufacture comprising:

a pair of HSV TK primers,

a pair of HSV TK probes, and

a donor fluorescent moiety and a corresponding acceptor moiety.

40. The article of manufacture of claim 39, wherein said pair of HSV TK primers comprise a first HSV TK primer and a second HSV TK primer, wherein said first HSV TK primer comprises the sequence

5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G, and wherein said second HSV TK primer comprises the sequence

5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C.

41. The article of manufacture of claim 39, wherein said first HSV TK probe comprises the sequence

5'-CGC GCG ACG ATA TCG TCT ACG TAC- 3' (SEQ ID NO:9), and wherein said second HSV TK probe comprises the sequence

5'-CGA GCC GAT GAC TTA CTG GCA GGT G- 3' (SEQ ID NO:10).

42. The article of manufacture of claim 39, wherein said pair of HSV TK probes are labeled with said donor fluorescent moiety and said acceptor fluorescent moiety.

43. The article of manufacture of claim 34, further comprising a label or package insert having instructions thereon for using said pair of HSV DNA polymerase primers and said pair of HSV DNA polymerase probes to detect the presence or absence of HSV in a biological sample.

44. The article of manufacture of claim 43, further comprising a label or package insert having instructions thereon for using said pair of HSV DNA polymerase probes to distinguish between HSV-1 and HSV-2.

45. A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein said cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if a nucleic acid molecule encoding HSV DNA polymerase is present in said sample, wherein said hybridizing step comprises contacting said sample with an HSV DNA polymerase probe, wherein said HSV DNA polymerase probe is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of fluorescence resonance energy transfer (FRET) between said donor fluorescent moiety and said corresponding acceptor fluorescent moiety of said HSV DNA polymerase probe,

wherein the presence or absence of FRET is indicative of the presence or absence of HSV in said sample.

46. The method of claim 45, wherein said amplification employs a polymerase enzyme having 5' to 3' exonuclease activity.

47. The method of claim 46, wherein said donor and acceptor fluorescent moieties are within no more than 5 nucleotides of each other on said probe.

48. The method of claim 47, wherein said acceptor fluorescent moiety is a quencher.

49. The method of claim 45, wherein said HSV DNA polymerase probe comprises a nucleic acid sequence that permits secondary structure formation, wherein said secondary structure formation results in spatial proximity between said donor and acceptor fluorescent moiety.

50. The method of claim 49, wherein said acceptor fluorescent moiety is a quencher.

51. A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein said cycling step comprises an amplifying step and a dye-binding step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if a nucleic acid molecule encoding HSV DNA polymerase is present in said sample, wherein said dye-binding step comprises contacting said HSV DNA polymerase amplification product with a nucleic acid binding dye; and

detecting the presence or absence of binding of said nucleic acid binding dye to said amplification product,

wherein the presence of binding is indicative of the presence of HSV in said sample, and wherein the absence of binding is indicative of the absence of HSV in said sample.

52. The method of claim 51, wherein said nucleic acid binding dye is selected from the group consisting of SYBRGreenI®, SYBRGold®, and ethidium bromide.

53. The method of claim 52, further comprising determining the melting temperature between said HSV DNA polymerase amplification product and said nucleic acid binding dye, wherein said melting temperature confirms said presence or absence of said HSV.

10066437-013182
201510-25499001